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Serum Auto Antibodies and Clinical/Pathological Features in German Shepherd Dogs with a Lupuslike Syndrome

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Thoren-Tolling, K. and L. Ryden: Serum auto antibodies and clinical/pathological features in German Shepherd dogs with a lupuslike syndrome. Acta vet. scand. 1991, 32, 15–26. – This study presents 8 dogs of German Shepherd breed (6 males, 2 females, 2–5 years of age at onset of the disease) with a lupus like syndrome characterized by febrile polyarthritis, wasting, nephropathy, cutaneous lesions and high positive titres of ANA (antinuclear antibodies) of speckled type. The serum autoantibodies were further characterized by double immunodiffusion against ENA (extractable nuclear antigen), ELISA for Histone antibodies (Histon fraction H-24A and H-3S), indirect IF on rat-liver sections, non treated and RNase/DNase digested sections for DNP/RNP antibodies, and smears of a hemoflagellate *C. luciliae* for antibodies vs doubbel strained DNA, (dsDNA). Thus, the high ANA titres in these dogs represent varying types of autoantibodies against nucleoproteins of both DNA and RNA nature, associated histone antigens and non-histone antibodies (RNA and Sm) as well. Rheumatoid Factor titres in serum from these dogs were low or negative.

Immunoglobulin deposits at dermo-epidermal junctions were demonstrated in some of the dogs with hyperkeratotic skin lesions. High concentration of serum-IgG was a constant finding in combination with anemia and in most cases leukopenia probably related to the chronic inflammatory process in these animals. Autoimmune hemolytic anemia (AIHA) or thrombocytopenia was not detected in these dogs.

canine lupus like syndrome; systemic lupus erythematosus (SLE); serum autoantibodies; antinuclear antibodies; rheumatoid factor; Coombs test; hyper-gammaglobulinemia; leukopenia; anemia.

Introduction

Canine rheumatoid arthritis (RA) and canine systemic lupus erythematosus (SLE) have been diagnosed with increased frequency in the last decades. At present the criteria for diagnosing these diseases are the same as employed by the American Rheumatism Association for diagnosis in man. This approach has gradually evolved into an accepted method of diagnosis in domestic animals, mainly dogs fulfilling some of the major clinical manifestations in these types of disease. For SLE; polyarthritis, skin lesions, weight loss, lymphadenopathy, hypergammaglobulinemia, nephropathy, myositis, pneumonitis, fever, positive titres af Antinuclear antibodies (ANA), hemolytic anemia and thrombocytemia are major criteria. For RA corresponding criteria are polyarthritis with stiffness and soft tissue swelling, in chronic cases subchondral erosions and focal calcifications, positive Rheumatoid

factor (RF) titres, hypergammaglobulinemia etc (*Amer. Rheum. Assoc.* 1973, *Tan et al.* 1982, *Grindem & Johnson* 1983, *Scott et al.* 1983, *Halliwell* 1978). Other clinical manifestations are either difficult to assess or are not acceptable in dogs. These includes Raynauds phenomenon, photosensitivity, positive Wasserman test, or abnormal behavior/physiological changes. The predictive values of these or other guidelines for the correct diagnosis of canine SLE cannot be ascertained until more is understod about the pathogenesis of systemic collagenous diseases. Also subsets of the SLE syndrome have been suggested in man and dogs as well (*Monier et al.* 1980).

During a systemic survey for ANA we encountered dogs of German Shepherd breed with polyarthritis and a lupus like syndrome. Symptoms were characterized by polyarthritis of chronical nature with recurrent febrile attacks and severe wasting associated with high positive titres for ANA that gave a speckled type fluorescence pattern to rat liver nuclei. In this study clinical, pathological and laboratory characteristics were investigated in 8 of those cases and combined with a screening for certain autoantibodies versus nuclear antigens, manifestation of Rheumatoid Factor and red blood cell antibodies.

Material and methods

Dogs

Eight German Shepherds, six males and 2 females, 2–5 years of age were selected from referrals to the Animal Hospital at the Veterinary College, Uppsala, Sweden. The dogs suffered from polyarthritis with painfull, swollen and stiff joints, wasting, general depression, intermittent fever and signs of chronic inflammatory reactions (hypergammaglobulinemia, anemia, abnormal leukocyte and differential count etc). The dogs were treated with varying doses of corticosteroids more or less continually during hospitalization. Administration of antibiotics was performed at different intervals in some of the dogs. Blood for immunological and biochemical analyses was sampled before the onset of any treatment and then during the hospitalization period. Due to severe and aggravating symptoms affecting general conditions, lameness and joint disorders, 4 of the dogs were put to death by intravenous anaesthesia and necropsied after a period of 2–4 months of hospitalization (AX, KS, CL, LS).

ANA

ANA are autoantobodies directed against different nucleoproteins: DNA/RNA and associated surrounding protein structures, Deoxyribonucleoprotein (DNP) and ribonucleoprotein (RNP), Histone fractions, Sm (Smith), SS (Sjögrens Syndrome) antigens. Nuclear antibodies in general lack species specificity. Antinuclear antibodies were detected by immunofluorescence (IF) on rat liver sections. Serum samples from dogs were analyzed for ANA in dilutions of 1:20 and 1:100 in PBS (phosphate buffered saline) for screening purposes and further titrated by two-fold dilutions to their end-points until the IF pattern was diluted out. A diagnostic second antibody, rabbit-anti-dog-7S (gamma)globulin/IgG fraction/[Nordic Laboratories, Tilburg, Netherlands] conjugated with FITC (fluorescine isothiocyanate) on Celite (according to *Rinderknecht* 1960) was used. The tissue slides were examined in incident light in a Leitz Orthoplan fluorescence microscope (Leitz, Wetslar, West-Germany).

Extractable nuclear antigens (ENA)

Antibodies against extractable nuclear antigen (ENA) were detected by immunodiffu-

sion (Ouchterlony technique) in 1 % agarose (phosphate buffer, pH 7.0). ENA was prepared from calf thymus as described by *Sharp et al.* (1972). Human control sera positive for RNP, Sm-, SS-nuclear antigen and DNA-histon was obtained from the National Bacteriological Laboratory [SBL], Stockholm, Sweden and used as positive control samples.

Histone fractions

Histones are basic nucleoproteins and components of the nucleosome. Autoantibodies to histones have multiple specificities and often react with several major classes of histone polypeptide fractions (H1–H4).

An ELISA technique for histone antibodies was performed according to *Rubin et al.* (1983) and *Epstein et al.* (1986). Briefly ELISA plates were coated with Histone fractions H-2A and H-3S [Sigma Chemical Comp. St. Louis, MO, USA], 100 µg/ml in bicarbonate buffer, pH 9.6. After washing serum diluted 1:100 on PBS/Tween 0.05 % and 1 % BSA was added and the plates were incubated at 37°C for 2 h. After another wash the plates were incubated with anti-dog-IgG conjugated with Alkaline Phosphatase (ALP) [Cappel Laboratories, Organon-Technica-Cappel, Walvern, PA, USA] and NPP (Nitrophenyl phosphate) in carbonate buffer, pH 9.8, was used as substrate. The enzyme activity was measured at 405 nm.

DNA and RNA antibodies

Rat liver sections mounted on glass slides were digested in DN:ase or RN:ase, [Deoxiribonuclease and Ribonuclease type I, Sigma Chem. Comp. St. Louis, MO, USA] approximately 500 and 800 Kunitz Units respectively per tissue section for 10 min at 37°C and then rinsed in PBS for 3 × 5 min and fixed in acetone before screening of ANA.

A hemoflagellate, *Crithidia luciliae* was also used as a substrate for detection of antibodies against dubble-strained DNA (dsDNA) by an indirect IF technique. (*C. luciliae* was obtained from The National Bacteriological Laboratory, [SBL], Stockholm, Sweden). A FITC conjugated rabbit-anti-dog-7S gammaglobulin [Nordic Laboratories] was used as second antibody and serum samples were analyzed in 1:10 and 1:100 dilutions.

Rheumatoid Factor (RF)

RF is a group of heterogenous high molecular weight proteins mainly of IGM class (RF of IgG and IgA nature are also described in man) directed against altered native IgG probably bound to immuncomplexes.

IgM rheumatoid factor was measured by an ELISA technique as described earlier (*Thoren-Tolling* 1990). Briefly micro-plates for ELISA were coated with dog-IgG (10 µg/ml) [Sigma Chemicals] in carbonate buffer, pH 9.6. After washing, patient serum diluted 1:100 in PBS/tween 0.05 %/ + 0.5M NaCl was added and serum-RF was detected by an ALP conjugated second antibody (rabbit-anti-dog-IgM/Fab2 fragment/), [Cappel Laboratories]. Furthermore a Latex agglutination technique was performed by use of a latex reagense for canine RF [Canine Rheumatoid Factor test kit, Synbiotics, San Diego, CA, USA] on heat inactivated serum samples.

Ig deposition in skin

Detection of immunoglobulin deposits at the dermo-epidermal junction and in the dermis was performed by immunfluorescence microscopy using FITC-labelled anti-dog-IgG [Cappel Laboratories] on frozen (–70°C) sections of skin biopsies.

Serum-Immunglobulins

IgG was estimated by nephelometric assay [Kallestad Nephelometer, Kallestad, Inc., Chaska, MN, USA] by use of rabbit-anti-dog-IgG/IgG fraction/, [Cappel Laboratories]. Serum was diluted 1:100 or 1:200 in PBS pH 7.4 with polyethylene glucol (PEG) 3 % added, and after addition of antiserum diluted in the same PBS/PEG buffer, the IgG concentration was registered as Light Scattering Units (LSU). All buffers were filtered through a 0.45 nm millipore filter before use for standardization of the nephelometric assay.

Coombs test for detection of autoantibodies against red blood cells was performed on fresh red blood cells according to standard procedures (*Schalm et al.* 1975). Commercially produced Coombs serum (anti-dog-IgG + C3), [Miles Inc. Kanakee, IL, USA] was used.

Hematology and clinical biochemistry

Hemoglobin and hematocrite estimation, erythrocyte, thrombocyte, leukocyte and differential cell count, total serum protein and serum enzyme activity; Aspartate Aminotransferase (ASAT,E.C.2.6.1.1.), Alanine Aminotransferase (ALAT,E.C.2.6.1.2.) and Alkaline Phosphatase (ALP,E.C.3.1.3.1.) were performed and measured according to standard procedures. Electrophoretic separation of serum and urine proteins was run on agarose gel, stained by Coomassie Blue and scanned in a densitometer [Corning 720 Densitometer, Corning Medical and Scientific Inc., Palo Alto, CA, USA]. Screening of proteinuria was performed also by precipitation in sulfosalicylic acid (10 %) and by use of reagense strips for albumin. Otherwise urine specimens were assayed according to standard procedures (reagense strips) and by microscopic examination for evaluation of urine sediments.

Results

Clinical and pathological findings

The clinical and serological observations in the dogs are summarized in Table 1. The most notable clinical signs are weight loss (often considerable), loss of appetite, depression and gradually impaired general condition accompanied by episodes of and later constant painfull soft tissue swelling of the joints. This is usually an early finding in the inflammatory process with a marked loss of function and later also atrophy of muscular tissue especially on upper limbs and loin. Polyarthritis seemed to attack joints on all limbs such as carpal, stiffle, coxofemoral, tarsal and elbow but also joints within the distal phalangeals. Joint involvement includes lameness which is sometimes difficult to associate with the involvement of a specific joint. Periarticular swelling appeared in exacerbations and remissions often with dogs being febrile during acute episodes. Articular lesions visible by X-ray radiography with soft tissue swellings, destruction of cartilage, slight erosive bone and changes and calcification, were seen in some of the dogs (AX and KS) whereas the polyarthritides were non erosive in the other dogs. Signs of polymalgia and polymyositis were also observed in these dogs.

The body temperature varied from periods of normal to peaks of 40–40.5°C. Other clinical signs were less consistant such as intermittent diarroea, alopecia-mostly patchy, seborreic skin and hyperkeratotic foot pads. Skin lesions appeared in 2 of the dogs (LS and AX) and were characterized by dark red erythematous, scaling lesions surrounded by a zone of hair loss resembling those changes normally associated with lupus diseases in man. No occular involvement (i.e. corneal ulceration, conjungivitis or scleritis) was observed in these dogs. The clinical treatment of the dogs described in this study in-

cluded symptomatic control of pain and joint stiffness with suitable analgesics and anti-inflammatory drugs. Administration of corticosteroids in high doses were often followed by some improvement of clinical signs and suppression of acute episodes, but in most cases the clinical response was weak. Antibiotics were also administrated in some dogs in order to suppress acute inflammatory reactions and usually in parallel with corticosteroid treatment.

In necropsied dogs, lymphadenopathy in peripheral lymph nodes and splenomegaly were common findings. Marked soft tissue swelling around affected joints, carpal, elbow, stifle, tarsal and shoulder joints were generally involved with hypertrophy and proliferation of the synovial membrane, proliferative synovitis and in some dogs (AX and KS) also subchondral erosions and slight focal calcifications were found. Kidneys were somewhat swollen with enlarged distinct glomeruli and basal membrane thickening. No pathological damage to pulmonary and myocardial tissues, endocarditis or cerebrospinal changes were observed in these dogs.

Clinical pathology and immunology

Abnormal laboratory findings were consistent with the systemic inflammatory process and included mild to moderate anemia and leukopenia often combined with lymphopenia. Lymphopenia and raised liver enzyme activities were also attributed to recent administration of corticosteroids. Leukocytosis with neutrophilia was seen occasionally in some dogs. Elevated plasma fibrinogen and ALP activity and in some cases a slight elevation in ALAT was also observed. Albuminuria was observed frequently in these dogs as demonstrated in Table 1.

Serum immunglobulins were constantly elevated in all dogs whereas the serum-albumin concentration was slightly lowered and consistent with the systemic inflammatory process. High titres of ANA were a general finding in these dogs with the highest titres observed prior to administration of corticosteroids. During corticoid treatment the ANA titres were lowered but positive titers were observed during the entire hospitalization period (Table 1). The fluorescence pattern was of speckled type and did not show any significant changes during the observation period.

Screening of antinuclear antibodies on RN:ase or DN:ase digested tissue sections is demonstrated in Table 1. It is seen that most of the ANA fluorescence in individual dogs was directed towards proteins of both DNA and RNA nature or proteins closely associated to the nucleic acids (DNP/RNP). Furthermore 4 of the dogs showed weak positive reactions for the *C. luciliae* test. It should be noted that positive reactions for *C. luciliae* in canine sera are faint and classified as weak positive reactions when compared to the strong positive immunfluorescence pattern for human control sera run simultaneously.

Antibodies vs RNP were detected by immunodiffusion against ENA i 7 of the dogs. One dog (GR) also showed precipitation reaction for Sm antigen. Weak positive immunodiffusion reactions for DNA-histone were observed in 3 of the dogs.

ELISA-histone fractions

Antibodies vs histone fraction H-2A was detected in 7 dogs in low to moderate titres. For fraction H-3S 4 dogs tested positive (Table 1).

Direct IF on skin biopsies from erythematous, hyperkeratotic areas revealed the presence of discrete deposits of IgG at the dermo-epidermal junctions in 3 of the dogs. IgG producing plasma cells were seen in 2

Table 1. Laboratory test results and clinical/pathological findings in 8 dogs of German Shepherd breed; LS, AX, GX, KS, CL, GR, LD and GG.

DOG	LS	AX	GX	KS	CL	GR	LD	GG
Sex (M/F)	M	M	F	M	M	M	M	F
Age (Years)	3	2	3	4	4	5	3	4
CLIN. SYMPTOMS								
Wasting	++	+	+	++	++	+	+	+
Poly arthritis	++	++	+	++	++	++	++	+
Body Temp. (C)	39–40	39–40.5	39–40	39–40	39–40.5	39–40	39–40	39–40
Diarrhea	+	(+)	0	+	0	+	0	0
Alopecia and hyperkeratosis	+	+	0	(+)	+	+	0	0
Foot pad reactions	+	+	0	+	+	+	0	0
CLIN. PATHOLOGY								
Hemoglobin (g/l)	–	–	–	–	–	–	–	–
Total leukocyte count	–	–	(+)	–	(+)	–	(–)	n
Differential count left <—> right shift	<—	<—	—>	<—	<—	<—	<—	n
Lymphocyte count	–	–	n	–	–	(–)	n	n
Fibrinogen	+	+	+	+	+	+	+	+
Albuminuria	++	+	+	(+)	(+)	++	(+)	0
Thrombocyte count	n	n	n	n	n	n	n	n
Coombs test	0	0	0	0	0	0	0	0
Serum-IgG (g/l)	35–40	25–30	25	25–30	30–35	25–30	20–25	20–25
SKIN (I.F.)								
Ig Deposition	plasma cells	DEJ	0	plasma cells	DEJ	DEJ	0	0
PATHOLOGY								
Lymph node hyperplasia	+	+	+	++	+	+	+	(+)
Splenic hypertrophy	+	+	+	+	+	NA	NA	NA
Synovial membrane hypertrophy	+	++	+	+	0	NA	NA	NA
Osteochondral lesions	0	+	+	+	0	NA	NA	NA

other dogs indicating non specific chronic dermatitis (Table 1).

The RF titres as measured by the ELISA were slightly above normal in 5 of the patients during hospitalization (Table 1) and the highest titres were observed prior to the administration of corticosteroids. This is seen also from the RF agglutination assay with mostly weak positive agglutination reactions in these dogs (Table 1).

Table 1. (Continued).

DOG	LS	AX	GX	KS	CL	GR	LD	GG
IMMUNOLOGY								
R F (ELISA)	+	0	+	0	+	(+)	0	+
R F (Latex aggl.)	+	0	+	0	++	+	0	+
ANA titre (I. F.)	1:800–1:3200	1:800–1:1600	1:400–1:800	1:400	1:400–1:800	1:800–1:3200	1:400–1:800	1:400 1:800
RNP ANA + DN/ase (I. F.)	0	+	+	0	++	+	+	++
DNP ANA + RN/ase (I. F.)	++	+	0	++	+	++	+	0
C. Luciliae (I. F.)	(+)	(+)	0	+	0	+	0	0
Histone fractions (ELISA) H-2A	+	+	+	0	+	++	+	+
H-3S	0	++	0	(+)	+	++	0	0
ENA (I. D.)								
RNP	(+)	+	+	0	+	+	+	+
Sm	0	0	0	0	0	+	0	0
SS	0	0	0	0	0	0	0	0
DNA-histone	0	+	0	0	0	+	(+)	0

Abnormal clinical/pathological signs: (+) weak, + significant, ++ severe.

Laboratory values:

– subnormal, (+) slightly above normal, + above normal, ++ significantly above normal, n :normal.

0: negative result, NA: Not analyzed.

ANA titres are expressed as end-point dilution of the serum samples.

Fluorescence reactions remaining after DN:ase or RN:ase digestion of the rat liver sections (ANA +DN:ase/RN:ase) are indicated as negative (0), weak (+), significant +, strong ++, fluorescence pattern.

The same symbols are used for the Crithidia luciliae test.

For immunological analyses, the following test methods are indicated;

ELISA = Enzyme linked immunosorbent assay.

Latex aggl. = Dog-IgG coated Latex agglutination assay

I. F. = Immunofluorescence technique

I. D. = Immunodiffusion (Ouchterlony technique)

D E J: dermi-epidermal junction

Discussion

SLE is a multisystem collagenic disease characterized by the formation of a variety of autoantibodies. Most characteristic of these are ANA directed vs nucleoproteins such as DNA, RNA, RNP, Histone related antigens, Sm, and SS nuclear antigens, red blood cell and thrombocyte autoantibodies.

Lewis et al. (1967) described a disease in the dog that closely resembled systemic lupus erythematosus in man. The syndrome included besides polyarthritis and glomerulinephritis, anemia and thrombocytemia of autoimmune nature. Several reports describing a similar syndrome in the dog have been presented since then (*Halliwell* 1978, 1982, *Scott et al.* 1983, *Grindem & Johnson* 1983) and many different canine breeds seem to be affected by this disease (*Scott et al.* 1983).

The cases presented in this study differ from earlier reports by the lack of autoantibodies against red blood cells and thrombocytopenia and the occurrence of febrile polyarthritis as major criteria. Furthermore the syndrome described in this study seems to affect German Shepherds in a high frequency. A similar rheumatic disorder predominantly observed in a population of German Shepherds was described by *Monier et al.* (1978, 1980), proceeding with febrile polyarthritis, severe wasting, positiv ANA titre and autoantibodies against RNP and Sm antigen.

Laboratory findings

Except for the rather frequent finding of proteinuria the urine is essentially normal in rheumatic diseases in man as well as in animals in SLE (*Scott* 1975, *Lewis* 1977, *Lipowitz & Newton et al.* 1976). Proteinuria both renal and post renal occurs for many reasons but is mostly associated with defective glomerular membrane filtration in these cases due to immunecomplex formation and/or autoantibodies deposited in the glomerular tissue (subendothelia/membranous glomerulinephritis). Renal amyloidosis may represent another cause of proteinuria in rheumatoid arthritis, and is also described in dogs (*Coles* 1974, *Newton et al.* 1976).

Other common laboratory findings consist of mild to moderate anemia and leukopenia, but leukocytosis and neutrophilia are also seen indicating fulminating and/or chronic inflammatory processes in these dogs.

The serum electrophoretic protein pattern in Rheumatic diseases is not specific and the most common feature is a lowered serum albumin concentration. Furthermore, elevation of alpha and beta globulin fractions (acute-phase globulins) is often seen in connection with the inflammatory processes going on whereas in more chronic cases significant hyper-gammaglobulinemia is the more characteristic findings (*Alexander et al.* 1975, *Monier et al.* 1980). In this study the elevated serum fibrinogen concentration was also consistent with the chronic inflammatory process as demonstrated in Table 1.

Serum enzymes

ASAT, ALAT, ALP and Creatine Phospho-Kinase (CK) are not constantly elevated in polyarthritis and other rheumatic disorder. Occasionally a slight increase in these enzyme activities is thought to be due to non specific tissue inflammatory processes affecting the liver (hepatocytic and bile canalicular) cell metabolism and muscular tissue as well (myalgia or myositis) often seen in canine RA (*Halliwell et al.* 1972, *Scott* 1975, *Hoffman* 1977) and other related chronical inflammatory conditions in domestic animals (*Dorner & Hoffman* 1974).

Auto-antobodies as criterion for rheumatic diseases

The absence of antinuclear antibodies is a strong evidence against SLE in man. On the

other hand ANA can be found in other rheumatic disorders mainly involving polyarthritis and is also reported in significant titres in canine rheumatoid arthritis (*Lewis & Borel* 1972, *Lipowitz* 1974, *Kass et al.* 1985). ANA is also as frequent finding in man with connective tissue diseases and is reported in about 15 % of RA patients (*Lipowitz & Newton* 1975, *Holborow & Reeves* 1977, *Jones* 1977). In a recent study approximately 10 % of the dogs from different breeds with varying rheumatic disorders came out positive for RF (*Thoren-Tolling* 1990), about 20 % of these cases were positive also for ANA (*Thoren-Tolling* unpubl.). In man almost 30 % of patients with SLE are reported having positive latex fixation test for RF (*Fye & Schack* 1987) whereas low titres of ANA are occasionally found in a wide variety of infectious/inflammatory disorders and also some neoplastic conditions in man and animals (*Werner et al.* 1983, *Tan* 1982, *Scott et al.* 1983). Since a positive ANA titre is a rather frequent finding there are grounds to postulate that the formation of immune complexes by ANA contributes to the immunpathology of these diseases in RA as well (*Holborow & Reeves* 1977). Little is known about these manifestations in the dog however.

Criteria for SLE

According to the criteria for SLE in man (*Amer. Rheum. Assoc.* 1973, *Tan et al.* 1982) and the major signs of canine SLE proposed by *Halliwell* (1978) and *Drazner* (1980) positive ANA test, polyarthritis (usually non-erosive), skin lesions, muscular involvement (myositis), profuse proteinuria often combined with hemolytic anemia, leukopenia and or thrombocytopenia are all diagnostic findings. The dogs in the present study showed low or negative RF titres which further indicates a systemic rheumatic disease as an etiological factor other than RA in these cases since canine RA is usually characterized by high titres of RF (*Halliwell* 1978, 1982, *Schultz* 1978). Deposition of immunoglobulins at the intradermal junctions in 3 of the dogs with erythematous and hyperkeratotic, scaling skin processes further support the involvement of an immun-mediated systemic disease in these dogs.

Antinuclear antibodies

The speckled type of immunfluorescense pattern is the most commonly observed type in man and animals and also the most varied in its description and normally reflects different types of autoantibodies (*Harmon* 1985, *Nakamura et al.* 1985). In man autoantibodies to non-histone nuclear proteins such as RNP as well as Sm and SS antigen typically demonstrates a speckled type of immunfluorescence. Clinically, human patients with variable large speckles appear to have an ill defined connective tissue disease (*Northway & Tan* 1972, *Harmon* 1985).

Extractable nuclear antigens generally demonstrate protein complexes consisting of non histone antigens and RNP. Sm and SS antibodies are thought to be associated to such ribonucleoproteins (*Fye & Schack* 1987, *Harmon* 1985, *Nakamura et al.* 1985). RNP and SS antibodies are frequently reported in SLE as well in other systemic rheumatic diseases and in Sjogrens syndrome whereas Sm antibodies are characteristic of SLE in man. Also, in the systemic type of disease described here, speckled type immunfluorescence representing non-histone antibodies (RNP and Sm) and histone components were detected as well. The titre of ANA in these dogs seems correlated to the severness of the disease and the highest levels of anti-histone antibodies were found in some of the most severe cases (Table 1). On the other hand the specificity of nuclear

antibodies seems not related to any specific clinical symptom in these animals.

DNA antibodies

The measurement of antibodies vs dsDNA in dog serum was performed by the *C. luciliae* test since the Farr Radio-immunoassay often used for measuring anti-DNA activity in human serum is unsatisfactory in the dog due to non specific DNA binding in this species as demonstrated in another report (*Zeromski et al.* 1984). The antibody titre vs dsDNA is normally correlated with the severity of human and murine SLE. However this contrasts with the low antibody titres detected in the dogs in this study and can therefore not be considered as an important criteria of the lupus like syndrome described here in German Shepherds. Also in other breeds anti-dsDNA activity is rare and only occasionally detected in canine SLE (*Costa et al.* 1984).

Autoimmine hemolytic anemia (AIHA), (as indicated by the Coombs test) and thrombocytopenia is frequent in the SLE syndrome in man (*Jones* 1977) and is often reported in dogs with this type of disease (*Halliwell* 1982, *Alexander* 1975, *Scott et al.* 1983). However the mild to moderate anemia seen in this study is not associated to AIHA and is more probably induced by the chronic inflammatory process in these dogs typically accompanied by hypergammaglobulinemia and other blood chemical abnormalities. Also, *Monier et al.* (1978 and 1980) were unable to demonstrate autoantibodies vs red blood cells in the German Shepherd lupus like syndrome. A predominance of female dogs (50–75 %) suffering from SLE has been reported by several authors (*Lewis* 1977, *Tizard* 1976, *Grindem & Johnson* 1983, *Alexander et al.* 1975) whereas *Monier et al.* (1980) reported a predominance of males in the lupus like syndrome in German Shepherds a finding indicated also in this study. The reason for the reported discrepances is not clear but variations in clinical symptoms from what has been described earlier for SLE in dogs may be of importance. These variations further suggest a classification of the syndrome described here as a systemic lupus like syndrome or a subset of SLE predominantly seen in German Shepherds.

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Sammanfattning

Autoantikroppar i serum och kliniska-patologiska förändringar hos Schäferhundar med ett lupus liknande syndrom.

Studien beskriver ett sjukdomssyndrom hos unga Schäfer-hundar (2–5 år vid debuten, 2 tikar och 6 hannar) uppvisande en systemisk reumatisk sjukdom liknande systemisk lupus erytematosus (SLE) med feber, polyartrit, avmagring, nefropati, hudförändringar och hög positiv ANA titer (Antinukleära antikroppar) med sk kornigt fluorescensmönster.

Serum från dessa hundar undersöktes också avseende förekomst av andra auto-antikroppar mot olika nukleära protein-strukturer; antikroppar mot ENA (Extractable nuclear antigen) bestämdes med immundiffusions teknik, Histon antikroppar analyserades med ELISA (H-2A och H-3S fraktioner), indirekt immunfluorescens teknik på DN/ase och RN/ase behandlade vävnadssnitt liksom utstryk av en flagellat, Crithidia lucilliae, användes för identifiering av antikroppar mot DNP/RNP respektive dubbelsträngat DNA (dsDNA). Autoantikroppar riktade mot antigen av såväl RNP som DNP natur påvisades hos dessa hundar, liksom mot associerade histoner och andre nukleära fraktioner (bla Sm-antigen). Reumatoid faktor titern i serum hos dessa hundar var genomgående låg eller negativ.

Hos några hundar med hyperkeratotiska hudförändringar påvisades immunglobulin deposition i övergångszonen mellan dermis och epidermis.

Samtliga hundar upvisade kraftigt förhöjda serum-IgG nivåer liksom anemi ofta förenat med leukopeni och troligen orsakat av den kroniska inflammations-processen vid detta sjukdomssyndrom. Autoimmun hemolytisk anemi eller trombocytopeni kunde däremot inte påvisas hos dessa hundar.

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